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Antifungal Susceptibilities among Different Serotypes of *Cryptococcus gattii* and *Cryptococcus neoformans*▽

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We measured antifungal activity against 128 cryptococcal isolates (86 of *C. neoformans* and 42 of *C. gattii*) to determine if differences in serotype susceptibility exist. Contrary to previous results, we found no serotype susceptibility differences. Isavuconazole, posaconazole, and voriconazole demonstrated excellent potency against each isolate and serotype, including isolates with reduced fluconazole susceptibilities.

Cryptococcosis is an invasive fungal infection most commonly caused by one of two species of encapsulated yeast, *Cryptococcus neoformans* and *Cryptococcus gattii*. Although previously classified as three *C. neoformans* varieties, proposed taxonomic changes have redefined *C. neoformans* and *C. gattii* as distinct species consisting of five serotypes: *C. neoformans* var. *grubii* (serotype A), *C. neoformans* var. *neoformans* (serotype D), the hybrid serotype AD, and *C. gattii* (serotypes B and C) (9). Immunocompromised patients are most commonly infected with serotypes A or D, while *C. gattii* has emerged as an important cause of infection for immunocompetent hosts, as illustrated by the recent outbreak on Vancouver Island, BC, Canada (6).

The incidence of cryptococcal meningitis has fallen after the introduction of antiretroviral therapy, although immunosuppressed populations remain at risk. Existing guidelines published by the Infectious Diseases Society of America do not define treatment differences by *Cryptococcus* spp., and for severe or central nervous system infections, a combination of amphotericin B and flucytosine remains the standard of care (14). However, previous reports of infections caused by *C. gattii* illustrate significant differences in the epidemiology, susceptibility patterns, chronicity of infection, and higher frequency of neurosurgical intervention than infections caused by *C. neoformans*; this is likely due to the propensity of *C. gattii* to form cryptococcomas (7, 17). These species' differences have prompted the search for antifungal agents with greater activity against *C. gattii*. Our objective was to measure the in vitro activities of antifungals against various cryptococcal serotypes to determine if differences in susceptibility exist. We also sought to determine the activity of the new extended-spectrum triazole, isavuconazole (formerly BAL4815), against *C. gattii*, as in vitro evaluation of this agent against a significant number of serotype B and C isolates has not previously been reported.

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A total of 128 *Cryptococcus* isolates were evaluated, including 86 isolates of *C. neoformans* (28 of serotype A, 25 of serotype D, and 33 of the hybrid AD serotype) and 42 isolates of *C. gattii* (30 of serotype B and 12 of serotype C), and were collected from both clinical and environmental sources from the United States, Australia, France, Denmark, Italy, Thailand, and the Democratic Republic of the Congo. Isolates were subcultured at least twice on Sabouraud dextrose agar (Remel, Inc., Lenexa, KS) prior to in vitro susceptibility testing. The previously available Crypto Check kit (Iatron Laboratories, Tokyo, Japan) was discontinued in 2004, so serotype identification of all isolates in this study was performed using a previously validated multiplex PCR method (4) and species were confirmed by growth/lack of growth on canavanine-glycine-bromothymol blue medium (8).

Susceptibility testing was done by broth microdilution in accordance with the CLSI M27-A2 methodology (12). Antifungals were obtained from the manufacturers as follows: isavuconazole (Basilea Pharmaceutica Ltd.), fluconazole (Pfizer), posaconazole (Schering-Plough), voriconazole (Pfizer), flucytosine (ICN Pharmaceuticals), and amphotericin B deoxycholate (Bristol-Myers Squibb). Stock solutions were prepared in dimethyl sulfoxide (isavuconazole, voriconazole, and amphotericin B), polyethylene glycol (posaconazole), or water (fluconazole and flucytosine) and were further diluted in RPMI 1640 medium (Sigma, St. Louis, MO) buffered to a pH of 7.0 with morpholinepropanesulfonic acid. Aliquots of each agent (0.1 ml) at 2× concentrations were dispensed into 96-well microdilution trays. Inocula containing 0.5×10^3 to 2.5×10^3 cells/ml were added, and trays were incubated at 35°C. Final antifungal concentrations ranged from 0.015 to 8 μg/ml for isavuconazole, posaconazole, voriconazole, and amphotericin and from 0.25 to 64 μg/ml for fluconazole and flucytosine.

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TABLE 1. MIC range, GM, and MIC_{50} and MIC_{90} values for antifungal agents against all *C. neoformans* ($n = 86$) and *C. gattii* ($n = 42$) isolates tested

Isolate and antifungal agent	MIC range	GM	MIC_{50}	MIC_{90}
Cryptococcus neoformans				
Amphotericin B	0.03–1	0.186	0.25	0.5
Flucytosine	0.25–16	3.6	4	8
Fluconazole	0.25–64	1.95	2	4
Posaconazole	<0.015–0.5	0.034	0.03	0.06
Voriconazole	<0.015–0.5	0.069	0.06	0.25
Isavuconazole	<0.015–0.5	0.023	<0.015	0.06
Cryptococcus gattii				
Amphotericin B	0.125–1	0.24	0.25	0.25
Flucytosine	0.06–16	1.97	2	8
Fluconazole	0.5–32	2.36	2	8
Posaconazole	<0.015–0.25	0.041	0.03	0.125
Voriconazole	<0.015–0.5	0.098	0.125	0.25
Isavuconazole	<0.015–0.25	0.026	0.03	0.06

The MICs for isavuconazole, posaconazole, voriconazole, fluconazole, and flucytosine were read as a 50% reduction in turbidity compared to growth control at 72 h. For amphotericin B, MICs were determined to have 100% inhibition relative to that of growth controls. *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were used as quality controls. Differences in geometric mean (GM) MICs were assessed by analysis of variance with Tukey's posttest for multiple comparisons.

The antifungal agents tested retained activity against all cryptococcal isolates. Table 1 summarizes the ranges and the MIC_{50} and MIC_{90} values against the *C. neoformans* and *C. gattii* isolates for each antifungal. The MIC_{50} and MIC_{90} values for isavuconazole (<0.015 and 0.06 μg/ml for *C. neoformans* and 0.03 and 0.06 μg/ml for *C. gattii*, respectively) were lower or equivalent to the corresponding values of all other antifungals. These results are consistent with those previously reported for *Candida* spp., with isavuconazole demonstrating lower MIC_{50} and MIC_{90} values than other agents (16). Similar potency was also observed for posaconazole and voriconazole. Excellent activity against isolates with intermediate susceptibility to fluconazole (MIC, 16 to 64 μg/ml) was also maintained for isavuconazole (0.06 to 0.5 μg/ml), posaconazole (0.125 to 0.5 μg/ml), and voriconazole (0.25 to 0.5 μg/ml).

When separated by serotype (Table 2), similar results were also observed. Isavuconazole, posaconazole, and voriconazole were active against each serotype ($MIC_{90} \leq 0.25$ μg/ml) and were more potent than the other agents tested when compared using geometric mean (GM) MICs ($P < 0.05$). Fluconazole and flucytosine were the least potent (MIC_{90}, 4 or 8 μg/ml against each serotype). Reduced flucytosine susceptibility in our study (23% MIC > 4 μg/ml) was higher than that previously reported (11%) (13). The clinical implications of this are unknown, as flucytosine is used in combination with amphotericin B and in vitro data have demonstrated synergy even against resistant isolates (MIC ≥ 32 μg/ml) (15).

Few differences were observed among the serotypes for each agent. Interestingly, no significant differences in potency were found for serotype B or C (*C. gattii*) compared to those of the other serotypes. This is consistent with the largest published

TABLE 2. GM, MIC range, and MIC_{50} and MIC_{90} values by serotype[a]

Serotype	Amphotericin B				Flucytosine			Fluconazole			Posaconazole			Voriconazole			Isavuconazole		
	GM	MIC range	MIC_{50}	MIC_{90}	GM	MIC range	MIC_{50}	MIC_{90}	GM	MIC range	MIC_{50}	MIC_{90}	GM	MIC range	MIC_{50}	MIC_{90}	GM	MIC range	MIC_{50}	MIC_{90}
A	0.25	0.06–0.5	0.25	0.5	3.123	1–8	4	4	2.32	0.25–32	2	4	0.064	<0.015–0.5	0.06	0.25	0.112	<0.015–0.5	0.06	0.25	0.036	<0.015–0.5	0.03	0.125
D	0.156	0.03–1	0.125	1	3.204	0.5–8	4	8	1.32	0.25–64	1	8	0.021	<0.015–0.125	<0.015	0.06	0.045	<0.015–0.5	0.03	0.25	0.019	<0.015–0.06	<0.015	0.03
AD	0.174	0.06–0.5	0.25	0.25	4.443	0.25–16	4	8	2.269	0.25–8	2	4	0.029	<0.015–0.06	0.03	0.06	0.064	<0.015–0.25	0.06	0.125	0.018	<0.015–0.06	<0.015	0.03
B	0.239	0.125–1	0.25	0.25	1.66	0.06–16	2	4	2.406	0.5–32	2	8	0.042	<0.015–0.25	0.03	0.125	0.098	<0.015–0.5	0.125	0.25	0.029	<0.015–0.25	0.03	0.06
C	0.25	0.125–0.5	0.25	0.5	2.997	1–8	2	8	2.245	0.5–4	2	4	0.038	<0.015–0.25	0.03	0.06	0.098	0.03–0.25	0.125	0.25	0.021	<0.015–0.125	<0.015	0.03

[a] Shown are 28 isolates of serotype A, 25 isolates of serotype D, 33 isolates of the hybrid AD serotype (86 of *C. neoformans*), 30 isolates of serotype B, and 12 isolates of serotype C (42 of *C. gattii*).

series evaluating species-specific MICs that reported no differences among serotypes (2, 11, 19). Other studies have described serotype-specific differences in antifungal potency (1, 3, 5, 10, 18, 21), raising concerns that infection with *C. gattii* may be met with a slower response to therapy (11), yet no consistent or predictable differences in antifungal activity were reported.

In this study, posaconazole, voriconazole, and the new triazole, isavuconazole, demonstrated excellent in vitro activity against all *Cryptococcus* serotypes tested, including isolates with reduced fluconazole susceptibility. This suggests extended-spectrum triazoles may be alternative treatments when similar isolates are encountered in clinical practice. However, clinical data are needed to confirm these results.

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